

The Evolving Role of Genetics in Reproductive Medicine

Paul R. Brezina, MD, MBA^{a,b,c,*}, William G. Kearns, PhD^{d,e}

KEYWORDS

- Genetics • Reproductive • Preimplantation genetic screening
- Preimplantation genetic diagnosis • Preimplantation • Chorionic villus sampling
- Amniocentesis • Cell-free DNA

KEY POINTS

- Genetics is increasingly being applied to reproductive medicine for both diagnosis and treatment.
- Preconception genetic testing, including testing for carrier states of conditions such as cystic fibrosis and hemoglobinopathies, will be increasingly used. There is an increasing trend to de-emphasize race to determine the appropriate testing.
- Antenatal genetic testing includes time-tested diagnostic evaluations such as chorionic villus sampling and amniocentesis. In addition, newer minimally invasive testing modalities are currently being developed and applied. The application of these tests is likely to increase in the future.
- Preimplantation genetic testing, including testing for specific genetic diseases and aneuploidy, is the practice of analyzing a biopsied sample of an egg or embryo obtained through in vitro fertilization. The ever improving accuracy and growing applications associated with this technology will likely lead to increased utilization.

Continued

Pertinent Disclosures and Conflicts of Interest: None.

Sources of Funding: None.

Contributors: P.R. Brezina primarily searched the literature. P.R. Brezina wrote the first draft of the article; W.G. Kearns edited the article and M.A. Kutteh advised on the content of the article, assisted in the literature search, and contributed to the writing of the article. P.R. Brezina is guarantor.

^a Reproductive Endocrinology and Infertility, Fertility Associates of Memphis, 80 Humphreys Center, Suite 307, Memphis, TN 38120, USA; ^b Department of Obstetrics and Gynecology, Vanderbilt University Medical Center, B-1100 Medical Center North, Nashville, TN 37232, USA; ^c Department of Surgery, MS133, Room B3019, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105-3678, USA; ^d Department of Gynecology and Obstetrics, Johns Hopkins Medical Institutions, Phipps 264, 600 North Wolfe Street, Baltimore, MD 21287, USA; ^e Preimplantation Genetics, The Center for Preimplantation Genetics, Lab-Corp, 15001 Shady Grove Road, Suite 200, Rockville, MD 20850, USA

* Corresponding author. Reproductive Endocrinology and Infertility, Fertility Associates of Memphis, 80 Humphreys Center, Suite 307, Memphis, TN 38120, USA.

E-mail address: pbrezina@fertilitymemphis.com

Obstet Gynecol Clin N Am 41 (2014) 41–55

<http://dx.doi.org/10.1016/j.ogc.2013.10.006>

obgyn.theclinics.com

0889-8545/14\$ – see front matter © 2014 Elsevier Inc. All rights reserved.

Continued

- Genetic analysis following fetal demise is now capable of evaluating the genetic karyotype of products of conception without growth in culture, through microarray technology. In addition, certain microarray evaluations are capable of ruling out maternal cell contamination. These advances may improve the diagnosis and future treatment of women suffering from failed pregnancy.
- It is incumbent on clinicians, however, to ensure that these interventions are used in a responsible, equitable, and ethical manner.

INTRODUCTION

At its core, reproductive medicine attempts to explain how human life is created and how it develops throughout pregnancy. Based on this understanding, therapies are developed and used to maximize outcomes. Specifically, increased pregnancy rates, decreased incidence of obstetric complications and miscarriage, and the avoidance of fetuses affected by birth defects or other deficiencies are the stated goal of much of the current research in reproductive medicine. The role of genetic testing to guide medical decision making in this regard is sizable and will likely continue to grow in the future.

Genetic evaluations within reproductive medicine may be subdivided into 4 main categories:

1. Preconception genetic testing: The genetic evaluation of prospective parents before pregnancy
2. Antenatal genetic testing: The genetic evaluation of women who are currently pregnant to determine the genetic makeup of the developing fetus
3. Preimplantation genetic testing: The genetic evaluation of an embryo, before uterine transfer, via an embryo biopsy during an in vitro fertilization (IVF) procedure
4. Genetic analysis following fetal demise: The genetic evaluation of the products of conception following a failed pregnancy

This article outlines each of these broad categories and describes the current appropriate applications of these technologies.

PRECONCEPTION GENETIC TESTING

Preconception genetic testing is the genetic evaluation of prospective parents before pregnancy (**Box 1**). Many individuals have a specific family history of certain genetic disorders, but many may also be at risk for unknown genetic diseases. Preconception genetic testing can be based on a couple's ethnicity or the medical history of a genetic

Box 1**Preconception genetic testing**

- Many individuals may be unknown carriers of certain genetic diseases
- Preconception testing for a variety of genetic diseases is increasingly recommended in phenotypically normal individuals
- Technological advances have improved the accuracy and have decreased the cost of such testing
- Race is increasingly de-emphasized to determine appropriate testing panels

disease segregating in their families. Most genetic diseases identified because of ethnicity are autosomal recessive disorders (AR), and require 2 mutations to have the disease. For a fetus to be affected with an AR disorder, each parent must pass along a mutation and, therefore, the fetal risk is 25%. One example of an AR disorder is cystic fibrosis (CF). In fact the carrier rate for CF among all individuals in the United States is 1 in 37, but may be as high as 1 in 27 in certain ethnic groups.¹

Genetic diseases segregating in a patient's family can be autosomal dominant (AD), AR, or X-linked (XL). For AD disorders, if the fetus has the mutation the baby will have the disease. An example of an AD disorder is Huntington disease. XL disorders exist on the X chromosome, and can be XL dominant or XL recessive. In simplistic terms, most XL disorders are caused by unaffected carrier mothers passing along their mutant X-chromosome to their affected sons. An example of an XL disorder is fragile X.

Various modalities and technologies are used for preconception genetic testing, and these test for the most common, though not necessarily all, mutations associated with the genetic disease. Most testing technologies include direct mutation analysis by genotyping, duplication/deletion analysis, and DNA sequencing, and can use classic linkage analysis.

Preconception genetic testing for couples considering pregnancy specifically to determine the carrier status for certain genetic AR disorders has been available for some time.² The list of recommended AR inherited disorders to evaluate in the context of preconception evaluation is limited and is primarily based on the recommendations of 2 societies.^{3,4} In addition, these societies often differ in exactly which tests are recommended.

Common conditions currently tested in a preconception genetic evaluation based on ethnicity include hemoglobinopathies (sickle cell trait, C trait, thalassemia trait, hemoglobin E), CF, Tay-Sachs disease, Canavan disease, familial dysautonomia, mucopolidosis IV, Niemann-Pick disease type A, Fanconi anemia group C, Bloom syndrome, Gaucher disease, and spinal muscular atrophy.

In the past, most preconception genetic testing was tailored to patients according to their ethnic background, as the carrier rate for specific disorders is known to differ among various ethnic groups. As the population of pan-ethnic societies, such as the United States, continues to become increasingly interracial, such targeted testing is becoming increasingly problematic.^{3,4} A recent bulletin by the American Congress of Obstetrics and Gynecology (ACOG) recently noted when evaluating this subject that "it is becoming increasingly difficult to assign a single ethnicity to individuals."^{3,4} Consequently, many experts now recommend offering universal carrier genetic testing for many of these AR disorders within the context of preconception counseling to all patients, regardless of their ethnic background.

When a positive result is obtained for a genetic disorder, the specifics of this result and its clinical risk must be explained clearly to the patient either by their clinician or a genetic counselor. In addition, patients should be counseled on available strategies to minimize the risk of having an affected child, including preimplantation genetic diagnosis within the context of an IVF cycle. Referral to a reproductive endocrinologist or geneticist familiar with these technologies may be advised.

ANTENATAL GENETIC TESTING

A central focus of obstetric care for many years has been to identify fetal disorders during pregnancy. In the age of elective terminations following the *Roe v. Wade* United States Supreme Court decision (410 U.S. 113; 1973), information regarding the status

of the developing fetus has been used to guide medical decisions. In general, the baseline incidence of some type of birth defect is approximately 3%.⁵ For many of these defects, detailed ultrasonography and other methods are used to identify specific anatomic anomalies. However, in most instances these anomalies are associated with a euploid (normal) genetic complement (either 46,XX or 46,XY). Although there may also be specific genetic abnormalities associated with this myriad of birth defects in euploid fetuses, genetic evaluation is currently not the modality used to identify such problems. Therefore, the remainder of the discussion in this article focuses on the genetic evaluations used to identify aneuploid (abnormal) genetic complements in the developing fetus during pregnancy.

The Basics of Aneuploidy

Chromosomal aneuploidy, a condition in which either too many or too few chromosomes are present on any 1 of the 23 chromosome pairs, is a common occurrence in human reproduction (**Box 2**). During the process, ovulation and fertilization human oocytes (eggs) and sperm undergo a series of organized genetic separation events known as meiosis. A simplified view of one of these events is outlined in **Fig. 1**. In this scheme, a diploid (2 copies of a chromosome) is divided into 2; 1 represents an ovulated egg and the other a discarded polar body. This haploid (having one copy of each chromosome) egg then is fertilized by another haploid sperm, producing a diploid embryo having 2 copies of each chromosome, one from each parent. In reality, however, this is a very simplified and incomplete summary of this process. Maternal meiosis in fact includes 2 phases of meiosis.

This process of meiosis is complex. If there is an uneven split of the chromosome pairs during meiosis, as is shown in **Fig. 2**, the resulting egg either has 2 or no copies of the chromosome. Following fertilization with sperm, this may result in either an extra copy of a chromosome (trisomy) or only 1 copy, not 2 copies, of a chromosome (monosomy). There are 23 chromosome pairs, and a splitting error on any 1 of these chromosomes results in a chromosomal complement that is unbalanced and generally not compatible with life. In fact, the only aneuploidy errors that can be compatible with a live birth are errors in the number of sex chromosomes (eg, 45X, 47XXY, 47XY) and trisomy in chromosomes 13, 18, or 21. Of these trisomies, chromosome 21 (Down syndrome) is generally associated with fetal survival past 1 year of age, and is associated with a host of developmental and health problems.

The focus of fetal genetic testing has traditionally focused on identifying trisomies in 13, 18, or 21 or X-chromosome monosomy, because these errors are the only problems that could be associated with a live birth. Aneuploidy errors on other chromosomes, while common, generally result in a failed pregnancy early in the first trimester before traditional testing (such as sampling of chorionic villi or amniocentesis) can practically be performed. However, technologies are currently available

Box 2

Antenatal genetic testing

- Aneuploidy is common in human embryos
- Modalities for testing the genetic status of the human fetus in utero have existed for some time, and include chorionic villus sampling and amniocentesis
- Evaluation of maternal cell-free DNA is a minimally invasive testing modality that offers the ability to identify aneuploidy in many cases. The application of this test is likely to increase in the future

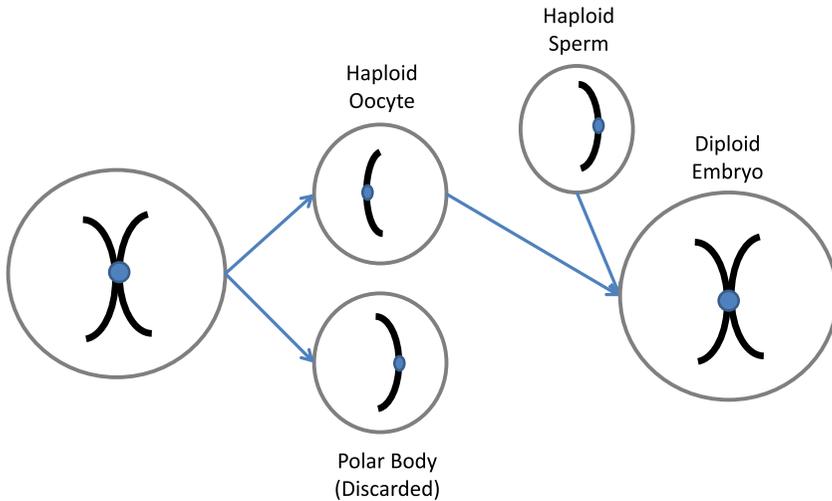


Fig. 1. Normal chromosomal division. This scheme depicts a normal meiotic division of one chromosome in which a diploid chromosome divides into haploid copies. One of these haploid copies joins with a haploid sperm, resulting in a diploid embryo.

that permit a more complete and, in some cases, less invasive fetal genetic evaluation. The following summarizes some of these interventions.

Chorionic Villus Sampling and Amniocentesis

Chorionic villus sampling (CVS) and amniocentesis are technologies that have been used for decades to determine whether aneuploidy or structural chromosome aberrations are present in the developing fetus. Often, these interventions are used after

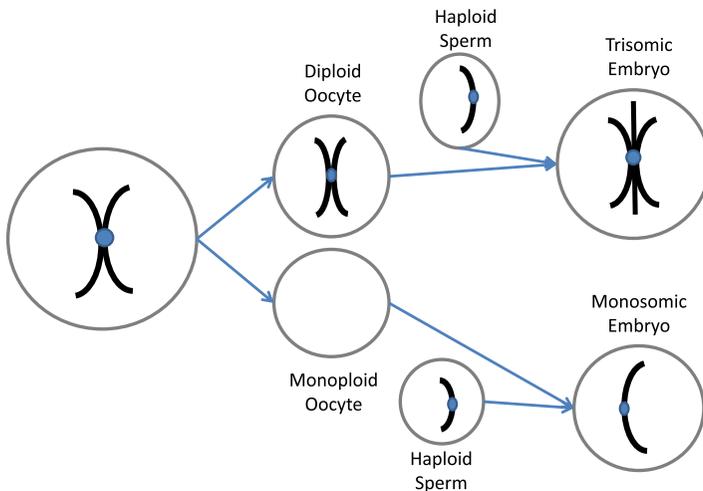


Fig. 2. Aneuploidy: meiotic nondisjunction. This scheme depicts the mechanism of meiotic nondisjunction whereby a diploid chromosome fails to divide into 2 haploid copies. After joining with a haploid sperm, this results in an embryo that has either 3 chromosomal copies (trisomy) or 1 chromosomal copy (monosomy).

there is some abnormality detected on ultrasonography or certain types of blood tests (hat screen for maternal proteins) commonly performed to determine the risk of aneuploidy in early pregnancy. In addition, women of advanced maternal age (>35 years) are often offered either CVS or amniocentesis. Both CVS and amniocentesis work by surgically obtaining and genetically evaluating fetal cells. In CVS, these cells are obtained by sampling chorionic villi via ultrasound-guided needle biopsy between 10 and 14 weeks of gestation.⁶ Amniocentesis is performed by needle aspiration of amniotic fluid around 15 weeks of gestation.⁶ At present, the samples obtained by either CVS or amniocentesis may be evaluated to generate a fetal karyotype or other targeted single-gene genetic testing as dictated by the clinical context.

However, CVS and amniocentesis have certain risks. First, the procedure of invasive sampling used with both these approaches is associated with pregnancy loss. The rate of pregnancy loss is estimated to be less than 1%, but is certainly not 0%.⁶ In addition, there is some level of maternal physical discomfort and stress associated with the performance of these procedures. Furthermore, CVS may provide spurious results in patients with a condition known as confined placental mosaicism, a condition whereby the placenta possesses a mixture of aneuploid and euploid cells while the fetus is euploid.

Cell-Free Fetal DNA Evaluation

It has been documented that nucleated cells derived from the fetal-placental unit can be detected circulating in maternal blood at a level of 1 to 6 cells per milliliter of maternal blood.^{7,8} More commonly observed in the maternal blood circulation are cellular fragments of DNA, generally thought to be placental in origin. These placentally derived DNA fragments actually comprise approximately 3% to 6% of all circulating cell-free DNA in maternal plasma.^{5,9,10} These fragments may be detected very early in pregnancy, but are not reliably present until later in the first trimester.^{7,8}

In 2007, Down syndrome (trisomy 21) was detected by genetic evaluation of these DNA fragments isolated from maternal blood.¹¹ Since this landmark case, the utilization of cell-free fetal DNA evaluation has become increasingly common. At present there are commercially available platforms that evaluate trisomies for chromosomes 13, 18, and 21 using this technique. However, these testing modalities are still considered a screen, and a diagnostic follow-up by CVS or amniocentesis is recommended.

Unlike CVS or amniocentesis, cell-free fetal DNA evaluation is noninvasive and is not associated with any rate of pregnancy loss resulting from the performance of the procedure. In addition, the test may be performed very early in pregnancy at approximately 10 weeks' gestation, well before when CVS or amniocentesis is possible. As this technology evolves and supportive data accumulate, an increased diagnostic role for this technology may be appropriate.

PREIMPLANTATION GENETIC TESTING

An IVF cycle consists of administering injectable gonadotropins to women and inducing controlled ovarian hyperstimulation whereby more than the usual number of ovarian follicles are recruited and matured.^{12,13} The oocytes within these follicles are then surgically harvested and inseminated with sperm. Typically resultant embryos are then grown in vitro until either 3 or 5 days of development, at which time the 1 or 2 best embryos are placed into the uterus and the remaining embryos are cryopreserved. It is possible to biopsy a cell(s) from developing embryos before embryo transfer, and to perform various genetic analyses that determine which embryos would be optimal for either embryo transfer or cryopreservation. Therefore, the

purpose of preimplantation genetic testing is to improve the likelihood of carrying a pregnancy to term and giving birth to a healthy baby.

When first introduced around 1990, preimplantation genetic testing was used exclusively to determine whether embryos harbored a specific genetic mutation that was known to exist from parental DNA analysis.^{14–16} This practice of evaluating embryos for a known parental genetic defect is known as preimplantation genetic diagnosis (PGD). As aneuploidy is the most significant reason for pregnancy failure, the technology of evaluating biopsied cells from embryos was then used to determine the ploidy status of embryos before uterine transfer.^{17,18} This evaluation of embryonic cells for aneuploidy from healthy parents, rather than a single genetic defect, is termed preimplantation genetic screening (PGS). PGD or PGS may be helpful to those who have experienced problems with infertility, recurrent pregnancy loss (miscarriages), or unsuccessful IVF cycles. PGD may also be useful to couples who are at risk for passing an inherited genetic condition on to their children (**Box 3**).

Preimplantation Genetic Diagnosis

As already discussed, PGD is the practice of evaluating embryos for a known parental genetic defect. The utilization of this technology is now widely accepted as appropriate in couples harboring a known genetic abnormality.¹³ The chief uses of PGD are outlined here.

Single-gene disorders

PGD for single-gene mutations diagnoses specific genetic mutations that are documented in the parents and segregating within their extended family. This goal is accomplished using polymerase chain reaction (PCR) to amplify the DNA of the chromosome where the gene of interest resides.¹⁹ DNA sequencing then identifies the specific gene

Box 3

Preimplantation genetic testing (PGT)

- PGT is achieved by obtaining a cellular biopsy from a developing human oocyte or embryo obtained via an in vitro fertilization cycle, evaluating the genetic composition of this sample, and using information gained from this process to determine optimal embryos for subsequent uterine transfer.
- PGT is performed by:
 - Obtaining cell(s) from either a developing embryo or oocyte
 - Genetic analysis of cells obtained at biopsy
- PGT is subdivided into 2 broad categories
 - Preimplantation genetic diagnosis (PGD)
 - The purpose of PGD is to prevent the birth of affected children from parents with a known genetic abnormality
 - PGD is widely acknowledged as acceptable for routine clinical application
 - Preimplantation genetic screening (PGS)
 - Attempts to identify aneuploidy in embryos to improve pregnancy success in certain patient populations
 - Parents with no identified genetic defect or disease
 - PGS remains controversial for routine application
- The results obtained by PGT may not always reflect the fetus' genetic composition.

mutation, and linkage analysis identifies surrounding markers used to determine recombination and whether the DNA of the sperm and oocyte is amplified.^{20,21}

Structural chromosome aberrations

In couples with recurrent pregnancy loss (RPL) and/or a documented balanced reciprocal/Robertsonian translocation (Fig. 3) or chromosomal inversion in one or both parents, PGD coupled with IVF has been shown to have some benefit in improving pregnancy and live birth rates.^{22–24} Fluorescence in situ hybridization (FISH) has traditionally been used to identify the presence of translocation imbalances in PGD translocation cases. FISH identifies chromosomal balances or imbalances, but is unable to rule out the presence of a balanced translocation chromosome. However over recent years microarrays have been increasingly utilized. Microarrays are able to simultaneously identify genetic imbalances caused by the parental translocation or inversion chromosome, and evaluate all 23 pairs of chromosomes from a single cell or cells. As with FISH, microarrays can only identify genetic balances or imbalances, and cannot differentiate the presence of a translocation chromosome from its normal counterpart.^{23,25}

Because of the complexities of PGD, all patients must be adequately counseled on the risks and limitations of PGD, preferably with the aid of a specialized physician, geneticist, or genetic counselor. Furthermore, antenatal genetic testing is still recommended in all patients undergoing PGS.¹³

Preimplantation Genetic Screening

PGS determines whether aneuploidy exists for any of the 23 pairs of chromosomes. The transfer of euploid embryos seems to play a significant role in implantation and fetal development. Determining which embryos are best has been a subject of much debate since the advent of IVF technology in the late 1970s. Traditionally the use of morphology (the visual appearance of embryos) has been the principal modality of choosing optimal embryos for uterine transfer.²⁶ However, the implantation rate per transferred embryo in most clinics rarely exceeds 40%.²⁷ Therefore, many investigators have for some time been searching to establish other diagnostic methods that are

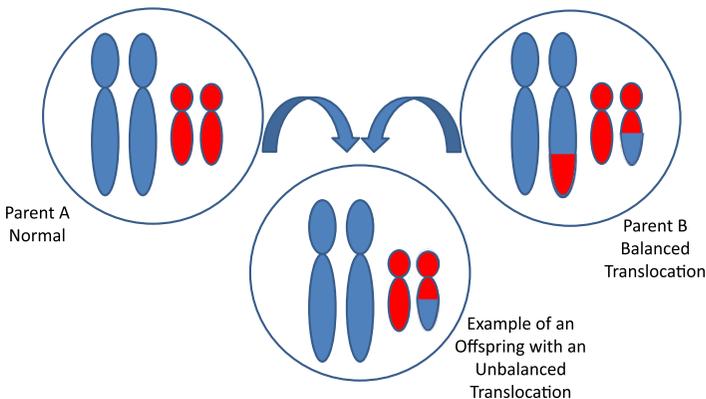


Fig. 3. Balanced parental translocations. This scheme depicts the mechanism by which parents who harbor a balanced chromosomal translocation may have offspring with unbalanced translocation errors. Of note, parents harboring balanced chromosomal translocations may also have offspring with different chromosomal combinations that may be phenotypically or genetically normal.

capable of determining embryo quality more accurately than morphology alone. PGS improves the implantation rate and delivery rate for RPL patients and, possibly, other clinical indications.

PGS, unlike PGD, has been and continues to be a controversial technology. Recent studies indicate that approximately more than 70% of all first-trimester miscarriages are the result of chromosomal aneuploidy.¹³ Because so many early miscarriages are due to aneuploidy, PGS seems to be a reasonable intervention to improve the efficiency with which euploid (chromosomally normal) embryos are selected for uterine transfer in IVF cycles. Classic studies have reported that first-trimester miscarriages resulting from aneuploidy are disproportionately concentrated on select chromosomes.^{28,29} These data are based on karyotype analysis of failed pregnancies that developed far enough to have tissue available for genetic analysis.^{28,29} Consequently, clinics performing PGS in the early days of the technology focused on detecting aneuploidy on only select chromosomes using FISH, which typically evaluates between 5 and 14 chromosome pairs rather than all 23 chromosome pairs.^{30,31} Traditionally, the PGS biopsy was exclusively performed at approximately 3 days of embryonic development following fertilization.^{30,31} Unfortunately, this approach failed to lead to improvements in clinical pregnancy rates, and this lack of efficacy was widely referenced following a landmark article by Mastenbroek and colleagues³² in the *New England Journal of Medicine*. Subsequently, similar articles cast further doubt on the benefits of PGS, and position statements from major medical societies formally discouraged its use.^{16,33,34}

Further research, however, elucidated several biological limitations that could explain the prior shortcomings of clinically applied PGS. First, studies have repeatedly documented that embryos at day 3 of development have high levels of mosaicism.^{35,36} A photograph of an embryo undergoing a day-3 biopsy is shown in **Fig. 4**. Mosaicism is a condition whereby a single developing embryo comprises more than 1 distinct genetic cell line. In other words, mosaic embryos may have euploid (normal) and aneuploid (abnormal) cell lines within a single embryo. Studies evaluating this phenomenon have concluded that as many as 50% of all embryos may be mosaic at day 3 of development.^{35,36} Consequently, a biopsy performed at day 3 of development may produce a result that is not representative of the entire embryo.¹³ It is clear that embryos developmentally change during differentiation to the blastocyst stage of development. Data have shown that mosaicism is greatly reduced in blastocysts, to approximately 5%.^{13,37}

Another limitation of traditionally performed PGS was the use of FISH for determination of chromosomal aneuploidies. FISH typically evaluates between 5 and 14 rather than all 23 chromosome pairs.³⁸ Recent studies have indicated that embryonic



Fig. 4. Cleavage-stage embryo. These photographs show an embryo at the cleavage stage. On the right is a photograph of a cleavage-stage biopsy.

aneuploidy occurs in clinically significant amounts in all 23 chromosome pairs.³⁹ Therefore, FISH is incapable of diagnosing many of the chromosomal abnormalities commonly found in developing embryos.

Realization of these 2 principal limitations have led many genetic laboratories to offer PGS using technologies evaluating the chromosomal status of all 23 chromosome pairs on cells obtained from differentiating blastocysts, typically reached by day 5 or day 6 of embryo development. A photograph of an embryo undergoing a day-5 biopsy is shown in Fig. 5. The clinical pregnancy rates using this approach have been reported to be markedly superior to the traditional approach of performing PGS.^{40,41} For example, a recent study evaluating more than 4500 embryos using determination of 23 chromosome pairs found clinical pregnancy rates in women suffering from RPL to be significantly improved in comparison with similar studies using FISH PGS.⁴⁰ In addition, pregnancy rates were further improved when 23-chromosome-pair evaluation PGS was performed on blastocyst stage embryos (day 5/6 of development), compared with when the biopsy was performed on embryos at day 3 of development.^{30,40,42} Similar results have been reported consistently by many clinics in the United States and around the world.^{30,42,43} This trend has led to a renewed interest in PGS, although it still remains to be determined whether PGS is an efficacious technology and which patient populations are best served by PGS.

Evaluation technologies using all 23 chromosome pairs are complex and differ in their experimental approach. The 2 most common technologies are microarrays and real-time PCR.¹³ Both of these technologies rely on obtaining embryonic DNA, amplifying this DNA, and evaluating the amplified product using microarrays or real-time PCR. This amplification process is a potential source of error, as failure to amplify the entire embryonic DNA genome could produce a false result. In addition, all amplification protocols must be performed under sterile conditions so as to avoid any exogenous contamination.

There are 2 types of microarrays, single-nucleotide polymorphism (SNP) and comparative genomic hybridization (CGH). SNP arrays directly evaluate ploidy by genotyping alleles on a dense chip of approximately 300,000 genetic markers.¹³ CGH arrays, by contrast, evaluate far fewer genetic markers and determine ploidy by comparing the clinical DNA sample with male and female reference DNA samples.¹³ Each of these microarray platforms have advantages and disadvantages. SNP arrays can identify loss of heterozygosity, consanguinity, and uniparental disomy whereas CGH arrays cannot, because of their ratio labeling protocol.

Significant advantages of CGH arrays are that that they may be performed in 12 hours and provide the IVF clinic the opportunity to transfer the embryos on day 6 of embryo development, potentially eliminate the need for an embryo freeze and a frozen embryo transfer, and can determine large clinically significant deletions or duplications. Real-time PCR is a molecular technique that determines the presence or



Fig. 5. Blastocyst-stage embryo. These photographs show an embryo at the blastocyst stage. The leftmost photo shows the herniation of trophoblast (TE) cells after the application of a laser to breach the zona pellucida. The next 2 photographs show the process of obtaining a sheet of TE cells that will be analyzed for preimplantation genetic screening.

absence of 3 to 5 chromosomal loci on the test sample DNA. It then quantitatively compares the chromosome copy number with a reference DNA sample. Real-time PCR can reliably determine aneuploidy in approximately 6 hours and can also provide IVF clinics the ability to transfer embryos on day 6 of development. One disadvantage of real-time PCR is the inability to determine structural chromosome aberrations.

Evidence for the Clinical Application of PGS

Studies from centers using PGS on day-5 and day-6 blastocysts show promising results, with transferred embryos generating pregnancy rates greater than 72%.^{30,40,42} However, a central criticism of the widespread use of PGS is a lack of randomized controlled trials that conclusively show the procedure to be beneficial. While some prospective trials currently do exist that support the application of PGS to improve pregnancy outcome, further studies are required before PGS will be more broadly accepted.^{13,43,44}

Despite the lack of support from professional societies and the lack of large, randomized controlled trials definitively demonstrating the benefits of the technology, PGS comprises the major part of all preimplantation genetic testing internationally, and is being increasingly used.⁴⁵ However, the patient population for which PGS may be appropriate is unclear at present.¹³ Many PGS clinics have traditionally recommended PGS for couples with risk factors believed to be associated with embryonic aneuploidy such as unexplained RPL, severe male factor, and advanced maternal age. However, in recent years many clinics have liberally expanded the use of PGS to many women without such risk factors. In fact, some clinics broadly recommend PGS to virtually all IVF patients as a strategy to improve pregnancy rates in all couples battling infertility. The debate surrounding the appropriate patient populations for PGS is currently in flux, and will likely be a source of debate for years to come.

Limitations of PGS

Despite the positive data emerging within the field of PGS, there are tangible technical and biological limitations to the technology. The limitations of FISH PGS evaluation and the use of biopsy taken from day-3 embryos are significant, as discussed earlier. In addition, technical limitations surrounding the use of both SNP and CGH arrays and real-time PCR may produce spurious results if not properly validated and experimentally performed.¹³

Perhaps the most significant source of error from PGS using 23-chromosome-pair evaluation on cleavage stage (day 3) or blastocysts (day 5/6 of development) is the presence of mosaicism within the developing embryo. Clearly it is not possible to perform genetic testing on every cell of an embryo, as this is not compatible with having a viable embryo available for uterine transfer. The best recommendation is to perform 23-chromosome-pair PGS on blastocysts.

The aforementioned limitations of PGS demand that patients be adequately counseled on its risks and limitations, preferably with the aid of a specialized physician, geneticist, or genetic counselor. Furthermore, antenatal genetic testing is still recommended in all patients undergoing PGS.¹³

New technologies associated with PGS are producing data suggesting that the procedure could be a valuable adjunct to assisted reproductive technologies in the future, to enhance pregnancy success in most patients. Defining the exact benefit conferred by PGS and determining exactly which patient populations could be best served by it, however, is currently controversial. In the near future, the field should commission large and high-quality studies that will attempt to answer these questions. Despite this lack of definitive data, however, PGS is increasingly being

Box 4**Genetic analysis following fetal demise**

- Aneuploidy is extremely common in embryogenesis and is responsible for the lion's share of pregnancy losses in the early first trimester
- Genetic analysis following fetal demise is now capable of evaluating the genetic karyotype of products of conception without growth in culture through microarray technology
- Certain microarray evaluations are capable of ruling out maternal cell contamination
- These advances may improve diagnosis and future treatment of women suffering from failed pregnancy

applied to patients with ever expanding clinical indications. Although there are few large, randomized controlled studies defining the benefits of PGS, clinical data emerging from many PGS laboratories around the world are encouraging. Therefore, the judicious use of PGS seems reasonable at present, as the preponderance of available current evidence suggests that some couples may derive benefit from this technology. Only with time will the role of PGS be clearly defined.

GENETIC ANALYSIS FOLLOWING FETAL DEMISE

As discussed earlier in the PGS section, chromosomal aneuploidy is extremely common in embryogenesis and is responsible for the lion's share of pregnancy losses in the early first trimester. However, determining whether aneuploidy existed in early first trimester losses has traditionally been difficult by routine karyotypic analysis, which requires the culture of cells followed by the generation of a G-banded karyotype. Failed cultures attributable to the presence of toxic substances and/or toxic cell products prohibit cell synchronization required for G-banding of metaphase chromosomes. Another important issue of routine karyotypic analysis of products of conception (POC) tissue is maternal cell contamination. Because of maternal cell contamination, with a 46,XX G-banded karyotype one cannot be certain that the analyzed cells are fetal or maternal in origin, and are therefore of little clinical value.

The introduction of SNP microarrays has dramatically changed the way first-trimester pregnancy losses are being evaluated. Arrays are capable of amplifying DNA samples from POC cells without requiring cell culture, thus permitting the analysis of small initial samples without the requirement of metaphase chromosome synchronization. Furthermore, SNP arrays determine genotypes, and if a maternal DNA sample is run with the POC tissue, one can differentiate a fetal 46,XX karyotype from a maternal 46,XX karyotype. Through these improvements, evaluation of first-trimester POCs through SNP microarrays has become an important component of the evaluation of RPL (**Box 4**).

SUMMARY

The past several decades have witnessed a dramatic increase in genetic diagnostics and therapeutic interventions offered to patients. Reproductive medicine is no exception to this statement. As our understanding of genetics and its relation to disease expands, there surely will be an ever expanding role for genetics within reproductive medicine. It is incumbent on clinicians, however, to ensure that these interventions are used in a responsible, equitable, and ethical manner.

ACKNOWLEDGMENTS

The authors thank Drs William Kutteh and Raymond Ke for their help in writing this article. They also extend gratitude to Michael A. Kutteh for his assistance in the preparation and submission of this article. His contributions are greatly appreciated, as he helped to facilitate the timely submission of this article.

REFERENCES

1. Strom CM, Crossley B, Buller-Buerkle A, et al. Cystic fibrosis testing 8 years on: lessons learned from carrier screening and sequencing analysis. *Genet Med* 2011;13(2):166–72.
2. Pletcher BA, Bocian M, American College of Medical Genetics. Preconception and prenatal testing of biologic fathers for carrier status. *American College of Medical Genetics. Genet Med* 2006;8(2):134–5.
3. ACOG Committee on Genetics. ACOG Committee Opinion No. 442: preconception and prenatal carrier screening for genetic diseases in individuals of Eastern European Jewish descent. *Obstet Gynecol* 2009;114(4):950–3.
4. American College of Obstetricians and Gynecologists Committee on Genetics. ACOG Committee Opinion No. 486: update on carrier screening for cystic fibrosis. *Obstet Gynecol* 2011;117(4):1028–31.
5. Bodurtha J, Strauss JF 3rd. Genomics and perinatal care. *N Engl J Med* 2012;366(1):64–73.
6. Simpson JL. Invasive procedures for prenatal diagnosis: any future left? *Best Pract Res Clin Obstet Gynaecol* 2012;26(5):625–38.
7. Go AT, van Vugt JM, Oudejans CB. Non-invasive aneuploidy detection using free fetal DNA and RNA in maternal plasma: recent progress and future possibilities. *Hum Reprod Update* 2011;17(3):372–82.
8. Ehrich M, Deciu C, Zwiefelhofer T, et al. Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting. *Am J Obstet Gynecol* 2011;204(3):205.e1–11.
9. Chiu RW, Akolekar R, Zheng YW, et al. Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. *BMJ* 2011;342:c7401. <http://dx.doi.org/10.1136/bmj.c7401>.
10. Rizzo JM, Buck MJ. Key principles and clinical applications of “next-generation” DNA sequencing. *Cancer Prev Res (Phila)* 2012;5:887–900.
11. Kido S, Sakuragi N, Bronner MP, et al. D21S418E identifies a cAMP-regulated gene located on chromosome 21q22.3 that is expressed in placental syncytiotrophoblast and choriocarcinoma cells. *Genomics* 1993;17(1):256–9.
12. Brezina PR, Benner A, Rechitsky S, et al. Single-gene testing combined with single nucleotide polymorphism microarray preimplantation genetic diagnosis for aneuploidy: a novel approach in optimizing pregnancy outcome. *Fertil Steril* 2011;95:1786.
13. Brezina PR, Brezina DS, Kearns WG. Preimplantation genetic testing. *BMJ* 2012;345:e5908.
14. ACOG Committee Opinion No. 430: preimplantation genetic screening for aneuploidy. *Obstet Gynecol* 2009;113(3):766–7.
15. Handyside A, Kontogianni EH, Hardy K, et al. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. *Nature* 1990;344:768–70.

16. Practice Committee of Society for Assisted Reproductive Technology, Practice Committee of American Society for Reproductive Medicine. Preimplantation genetic testing: a Practice Committee opinion. *Fertil Steril* 2008;90:S136–43.
17. Hassold T, Chen N, Funkhouser J, et al. A cytogenetic study of 1000 spontaneous abortions. *Ann Hum Genet* 1980;44:151–78.
18. Menasha J, Levy B, Hirschhorn K, et al. Incidence and spectrum of chromosome abnormalities in spontaneous abortions: new insights from a 12-year study. *Genet Med* 2005;7:251–63.
19. Verlinsky Y, Cieslak J, Freidline M, et al. Pregnancies following preconception diagnosis of common aneuploidies by fluorescent in-situ hybridization. *Hum Reprod* 1995;10:1923–7.
20. Laurie AD, Hill AM, Harraway JR, et al. Preimplantation genetic diagnosis for hemophilia A using indirect linkage analysis and direct genotyping approaches. *J Thromb Haemost* 2010;8:783–9.
21. Cirulli ET, Goldstein DB. Uncovering the roles of rare variants in common disease through whole genome sequencing. *Nat Rev Genet* 2010;11:415–25.
22. Kuliev A, Verlinsky Y. Current features of preimplantation genetic diagnosis. *Reprod Biomed Online* 2002;5(3):294–9.
23. Treff NR, Northrop LE, Kasabwala K, et al. Single nucleotide polymorphism microarray-based concurrent screening of 24-chromosome aneuploidy and unbalanced translocations in preimplantation human embryos. *Fertil Steril* 2011;95(5):1606–12.e2.
24. Otani T, Roche M, Mizuike M, et al. Preimplantation genetic diagnosis significantly improves the pregnancy outcome of translocation carriers with a history of recurrent miscarriage and unsuccessful pregnancies. *Reprod Biomed Online* 2006;13(6):869–74.
25. Du L, Brezina PR, Benner AT, et al. The rate of de novo and inherited aneuploidy as determined by 23-chromosome single nucleotide polymorphism microarray (SNP) in embryos generated from parents with known chromosomal translocations. *Fertil Steril* 2011;96(3):S221.
26. Brezina PR, Zhao Y. The ethical, legal, and social issues impacted by modern assisted reproductive technologies. *Obstet Gynecol Int* 2012;2012:686253.
27. Ajduk A, Zernicka-Goetz M. Advances in embryo selection methods. *F1000 Biol Rep* 2012;4:11.
28. Monni G, Ibba RM, Zoppi MA. Prenatal genetic diagnosis through chorionic villus sampling. In: Milunsky A, Milunsky JM, editors. *Genetic disorders and the fetus*. 6th edition. Oxford (United Kingdom): Wiley-Blackwell; 2010.
29. Kearns WG, Pen R, Graham J, et al. Preimplantation genetic diagnosis and screening. *Semin Reprod Med* 2005;23(4):336–47.
30. Schoolcraft WB, Fragouli E, Stevens J, et al. Clinical application of comprehensive chromosomal screening at the blastocyst stage. *Fertil Steril* 2010;94:1700–6.
31. Brezina PR, Kearns WG. Preimplantation genetic screening in the age of 23-chromosome evaluation; why FISH is no longer an acceptable technology. *J Fertiliz In Vitro* 2011;1:e103.
32. Mastenbroek S, Twisk M, Echten-Arends J, et al. In vitro fertilization with preimplantation genetic screening. *N Engl J Med* 2007;357:9–17.
33. Checa MA, Alonso-Coello P, Solà I, et al. IVF/ICSI with or without preimplantation genetic screening for aneuploidy in couples without genetic disorders: a systematic review and meta-analysis. *J Assist Reprod Genet* 2009;26:273–83.

34. Mastenbroek S, Twisk M, van der Veen F, et al. Preimplantation genetic screening: a systematic review and meta-analysis of RCTs. *Hum Reprod Update* 2011;17:454–66.
35. Harton GL, Magli MC, Lundin K, et al, European Society for Human Reproduction and Embryology (ESHRE), PGD Consortium/Embryology Special Interest Group. ESHRE PGD Consortium/Embryology Special Interest Group—best practice guidelines for polar body and embryo biopsy for preimplantation genetic diagnosis/screening (PGD/PGS). *Hum Reprod* 2011;26:41–6.
36. Munné S, Weier HU, Grifo J, et al. Chromosome mosaicism in human embryos. *Biol Reprod* 1994;51:373–9.
37. Brezina PR, Sun Y, Anchan RM, et al. Aneuploid embryos as determined by 23 single nucleotide polymorphism (SNP) microarray preimplantation genetic screening (PGS) possess the potential to genetically normalize during early development. *Fertil Steril* 2012;98(3):S108.
38. Harper JC, Sengupta SB. Preimplantation genetic diagnosis: state of the art 2011. *Hum Genet* 2012;131:175–86.
39. Brezina PR, Tobler K, Benner AT, et al. All 23 chromosomes have significant levels of aneuploidy in recurrent pregnancy loss couples. *Fertil Steril* 2012;97(3):S7.
40. Brezina PR, Tobler K, Benner AT, et al. In vitro fertilization (IVF) cycles and 4,873 embryos using 23-chromosome single nucleotide polymorphism (SNP) microarray preimplantation genetic screening (PGS). *Fertil Steril* 2012;97:S23–4.
41. Wells D, Alfarawati S, Fragouli E. Use of comprehensive chromosomal screening for embryo assessment: microarrays and CGH. *Mol Hum Reprod* 2008;14:703–10.
42. Forman EJ, Tao X, Ferry KM, et al. Single embryo transfer with comprehensive chromosome screening results in improved ongoing pregnancy rates and decreased miscarriage rates. *Hum Reprod* 2012;27:1217–22.
43. Scott RT Jr, Upham KM, Forman EJ, et al. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. *Fertil Steril* 2013. <http://dx.doi.org/10.1016/j.fertnstert.2013.04.035>.
44. Yang Z, Liu J, Collins GS, et al. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. *Mol Cytogenet* 2012;5:24.
45. Harper JC, Wilton L, Traeger-Synodinos J, et al. The ESHRE PGD Consortium: 10 years of data collection. *Hum Reprod Update* 2012;18:234–47.